Scientific Research Publishing

Advances in Microbiology, 2019, 9, 943-955
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Screening of Some Lactic Acid Bacteria Isolated from Selected Nigerian Fermented Foods for Vitamin Production

Anayochukwu Chibuike Ngene[1*], Chijioke E. Onwuakor[2], John C. Aguiyi[1], Virginia O. Ifeanyi[2], Chinedu Godspower Ohaegbu[2], Chukwuka Peter Okwuchukwu[2], Esther Garos Kim[3], John O. Egbere[4]

[1]Africa Center of Excellence in Phytomedicine Research and Development, University of Jos, Jos, Plateau State, Nigeria
[2]Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
[3]Department of Medical Microbiology, Faculty of Clinical Sciences, College of Health Sciences, University of Jos, Jos, Plateau State, Nigeria
[4]Department of Microbiology, Faculty of Natural Sciences, University of Jos, Jos, Plateau State, Nigeria
Email: *ngeneanayochukwu@yahoo.com

How to cite this paper: Ngene, A.C., Onwuakor, C.E., Aguiyi, J.C., Ifeanyi, V.O., Ohaegbu, C.G., Okwuchukwu, C.P., Kim, E.G. and Egbere, J.O. (2019) Screening of Some Lactic Acid Bacteria Isolated from Selected Nigerian Fermented Foods for Vitamin Production. *Advances in Microbiology*, **9**, 943-955.
https://doi.org/10.4236/aim.2019.911060

Received: September 17, 2019
Accepted: November 15, 2019
Published: November 18, 2019

Abstract

Human vitamin deficiencies still occur in many countries although most vitamins are present in a variety of foods, mainly because of malnutrition not only as a result of insufficient food intake but also because of unbalanced diets; this work screens some Lactic acid bacteria isolated from selected Nigerian fermented foods for vitamin (thiamine, riboflavin and niacin) production. Five lactic acid bacteria were isolated from selected Nigerian fermented foods (yoghurt, ogi, ogiri, ugba). The isolated lactic acid bacteria were identified based on cultural and biochemical characteristics. All the isolates were screened for thiamine, riboflavin and niacin production using microbiological assay. Thiamine, riboflavin and niacin produced were quantified. The five lactic acid bacteria isolated were *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus casei* and *Lactococcus lactis*. The most frequent isolate was *Lactobacillus plantarum* (100%) followed by *Lactobacillus caesi* (75%). *Lactobacillus brevis*, *Lactobacillus fermentum* and *Lactobacillus lactis* were the least isolates having (50%) occurrence each. *Lactobacillus plantarum* produced the highest amount of thiamine (5.8833 µg/100ml) and riboflavin (5.0400 µg/100ml) while *Lactobacillus lactis* had the least thiamine (5.2267 µg/100ml) and *Lactobacillus caesi* had the least riboflavin (4.8300 µg/100ml). *Lactobacillus casei* produced the highest niacin (1.6433 µg/100ml) while *Lactobacillus fermentum* had the least (1.2900 µg/100ml). This study reveals *Lactobacillus plantarum* as the most efficient thiamine and riboflavin producer while *Lactobacillus casei* produced the most niacin.

Keywords

Vitamin, Lactic Acid Bacteria, Fermentation, Thiamin, Riboflavin, Niacin

1. Introduction

It has been observed that lactic acid bacteria (LAB) is a large and heterogeneous group of Gram-positive bacteria characterized by a strictly fermentative metabolism with lactic acid as the major end product during sugar fermentation [1]. Apart from producing the lactic acid, LAB contributes to the flavour, texture and nutritional value of the fermented foods by the production of aroma components, modification or production of proteins and exopolysaccharides, and the production of nutritional components such as vitamins [2] [3]. Some lactic acid bacteria like Streptococcus and Lactobacillus strain are mostly used in food and pharmaceutical industries due to their healthful properties [3] [4] [5]. Many strains of LAB have the ability to synthesize some B vitamins such as riboflavin and niacin including folic acid in dairy products [6]. The use of vitamin-producing micro-organisms is thus a more natural and economically viable alternative than fortification with chemically synthesized pseudo-vitamins, and it would allow the production of foods with elevated concentrations of vitamins that are less likely to cause undesirable side effects. Lactic acid bacteria (LAB) are naturally present in a broad range of ecological niches such as foods and in the gastrointestinal and urogenital tract of animals, including humans. In addition to their technological important properties in food production, several studies have shown that LAB can confer beneficial properties to their hosts, in specific members of the genus Lactobacillus, reason for which these bacteria are the most commonly used probiotic micro-organisms [7]. Thus, they can later be defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [8]. Some probiotics health benefits include promotion of normal microbiota, prevention of food allergies and infectious diseases, reduction of cholesterol in serum, improved anticarcinogenic activity, stabilization of intestinal mucosal barrier, body immune adjuvant properties, alleviation of intestinal bowel disease symptoms and improvement in the digestion of lactose in intolerant hosts [9] [10] [11]. Besides probiotic LAB, particular strains of LAB are able to produce/release and/or increase specific beneficial compounds in foods. These functional ingredients are most-times referred to as nutraceuticals a term that was first given by Stephen DeFelice in 1989 to describe "a food (or part of a food) that provides medical or health benefits, including the prevention and /or treatment of a disease". These ingredients can be macronutrients, micronutrients (such as vitamins) or non-nutritive compounds that can be naturally present in certain foods or added when processing. The proper selection and exploitation of nutraceutical-producing micro-organisms is an interesting strategy to produce new foods with increased nutritional and/or health-promoting

properties [12]. Isolation of indigenous LAB from various fermented foods and other sources has been carried out by Indonesian researchers [13] [14] [15] [16] and has been screened for their health importance such as probiotics [17] [18], β-glucosidase producers [16] [19], functions as angiotensin-converting enzyme (ACE) inhibitor producer [20]; isoflavone and antioxidant properties [21]. In this study, indigenous lactic acid has been isolated again from selected Nigerian fermented foods and screened for its ability in synthesis of Thiamine, Riboflavin and Niacin production during fermentation.

Application of Lactic Acid Bacteria (Figure 1)

Aim

The broad aim of this work is to screen some Lactic Acid Bacteria isolated from selected Nigerian fermented foods for vitamin (Thiamine, Riboflavin and Niacin) production.

Objectives

- To isolate Lactic Acid Bacteria from selected Nigerian fermented foods;
- To characterize and identify the isolates;
- To screen these Lactic Acid Bacteria isolates for vitamin (Thiamine, Riboflavin and Niacin) production.

2. Materials and Method

2.1. Source of Materials

The test samples for sourcing lactic acid bacteria (LAB) includes yoghurt (fermented diary product), ogi (fermented maize, *Zea mays*), ogiri (fermented melon seed, *Citrullus vulgaris*) and ugba (fermented African oil beans (*Pantaclethra macrophylla*) were sourced randomly from the open market by random selection. Laboratory materials were sourced from Ceslab Analytical services, National Root and Crop Research Institute (NRCRI) Umudike where the research analysiss was carried out.

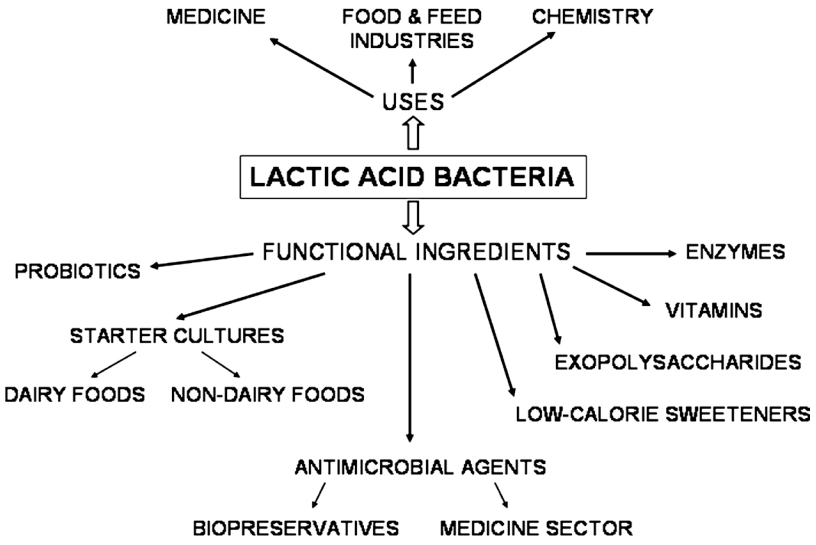

Figure 1. uses and functional ingredients of LAB [22].

2.2. Sample Preparation

Samples were collected into sterile universal bottles and were taken into the laboratory for microbiological examination. They were homogenized by adding 1 milliliter (ml) of yoghurt into 9 ml of sterile peptone physiological saline solution, 1 gramme (1 g) of ogi, ugba and ogiri was homogenized respectively into different 10 ml of sterile peptone physiological saline solution [23].

2.3. Media Preparation

According to manufacturers' instruction, 67.1 g of de Man Rogosa Sharpe (MRS) medium was dissolved in 1000 ml distilled water. Gently heated to dissolve the medium completely, sterilized by autoclaving at 15 psi (121˚C) for 15 minutes, cooled at room temperature prior to dispense into sterile Petri dish plates. MRS broth was prepared by dissolving 67.1 g of the medium in 1000 ml distilled water and filter to remove the agar prior to sterilization.

2.4. Isolation of Lactic Acid Bacteria

Serial dilutions of each of the prepared sample were carried out. 0.1 ml of the third (10^{-3}) diluents was inoculated into MRS agar by spread plate method and incubated at 37˚C for 48 hours. After incubation, colonies were purified by successive streaking on MRS agar and maintained on MRS agar slant, stored at 4˚C and subcultured at 14 days interval [24].

2.5. Morphological Identification of Bacterial Isolates

Gram Staining

Gram staining of the isolates was carried out to see whether the isolates were gram negative or gram positive organisms as follows: the smear of the organism was made on a clear grease free slide and fixed. It was covered in the crystal violent stain for 60 seconds. The stain was washed off with distilled water. The smear was covered with Lugol's iodine for 60 seconds and then washed with distilled water. It was decolourized rapidly (few seconds) with acetone, washed immediately with clean water, it was covered with saframin stain for 2 minutes and washed with distilled water. The back of the stained slide was wiped with tissue paper and placed in a draining rack for the smear to air dry. The stained slide was examined microscopically using oil immersion objective (×100) [25].

Biochemical parameters.

For further identification of the isolates the following biochemical tests were carried out according to [26].

Catalase test

Two milliliters (2 ml) of hydrogen (3%) was poured into a test tube. Using a sterile wooden stick, a good growth of the isolates was removed and immersed into the hydrogen peroxide solution. No release of bubbling was observed. This showed negative (no catalase produced). Immediate bubbing showed positive (catalase produced).

Oxidase test

Using a piece of sterile wooden stick, a colony of the isolates was removed and smeared on oxidase paper strips. Development of a blue-purple colour within 10 seconds showed positive while no blue-purple colour showed negative test.

Methyl red test

Colonies of the isolates were incubated into 0.5 ml sterile glucose phosphate peptone water, incubated anaerobically at 37˚C for 24 hours. A drop of methyl red solution was added. A bright red colour indicating acidity showed positive while a yellow colour showed negative.

Voges-Proskauer (VP) test

The isolates were inoculated into 2 ml of sterile glucose phosphate peptone water medium and incubated anaerobically at 37˚C for 48 hours. A very small amount of creatinin was added to the culture and mixed. About 3 ml of the sodium hydroxide (NaOH) reagent were also added and mixed very well. The bottle cap was removed and the preparation left to stand for 1 hr at room temperature, slow development of a pink-red colour was indicative of a positive VP test.

Indole and motility test

Using a sterile straight wire loop, 5 ml of sterile MIU (motility indole urea) medium was incubated with the isolates. An indole paper strip was placed in the neck of the MIU tube above the medium. The tube was covered and incubated anaerobically at 37˚C over night. Reddening of the lower part of the strip showed indole positive while no reddening showed negative. For motility, spreading of turbidity from the stab-line or turbidity throughout the medium showed positive while no turbidity showed negative.

Citrate Utilization

The isolates were inoculated into peptone water medium, incubated anaerobically at 37˚C for 24 hours. Using sterile wire loop, the broth culture of the isolates was inoculated into 4 ml of sterile koser's citrate medium. The incubated medium was inoculated anaerobically at 37˚C for up to 4 days with the screw loosened during incubation. The inoculated medium was checked daily for growth. Turbidity and blue colour showed a positive citrate test.

Sugar fermentation test

The isolates were inoculated into MRS broth, incubated anaerobically at 37˚C for 24 hours, using a sterile pastuer pipette, 4 ml of prepared MRS broth with 2ml of 10% sugar (glucose, fructose lactose, mannose, sucrose, maltose and galactose) and Durhan tube (inverted) was inoculated with overnight broth of the isolates and incubated anaerobically at 37˚C for 24 hours. Sugar fermentation, which were indicated by change in the colour of the medium and gas production in the Durhan tubes were observed.

Growth in 4.5% NaCl and 6.5% NaCl

The isolates were inoculated into prepared MRS broth with 4.5% NaCl and 6.5% NaCl respectively, incubated anaerobially at 37˚C for 24 hours. Turbidity showed positive (growth), no turbidity showed negative (no growth).

Growth at 15˚C and 45˚C

The isolates were inoculated in to MRS broth, incubated anaerobically at 15˚C and 45˚C respectively for 24 hours. Turbidity showed positive (growth) no turbidity showed negative (no growth).

2.6. Determination of Thiamine (Vitamin B_1)

The spectrophotometric method describe by Okwu (2004) was used [27]. A measured volume (5 ml) of each prepared inoculums broth was homogenized with 50 ml of 1 ml ethanolic sodium hydroxide and the homogenate was filtered to obtain the filtrate used for the analysis. An aliquot (10 ml) of the filtrate was treated with equal volume of 0.1 ml $K_2Cr_2O_7$ solution in a flask. Meanwhile, standard thiamine solution was prepared and diluted to a chosen concentration of (0.05). An aliquot of standard thiamine solution was also treated with 10 ml of the dichromate solution ($K_2Cr_2O_7$) in a separate flask while a regent blank was set up by treating 10 ml of the ethanol sodium hydroxide with the potassium dichromate solution. The absorbance of the sample and the standard solution were measured in a spectrophotometer at a wavelength of 360 nm with the reagent blank used to calibrate the instrument at zero. The thiamine content was calculated using the formula below:

$$\text{Thiamine } \mu\text{g}/100\text{ml} = \frac{100}{V} \times au \times \frac{C}{as} \times VF \times \frac{D}{va}$$

where: V = Volume of the inoculums broth

au = Absorbance of sample

as = Absorbance of standard sample

C = Concentration of standard thiamine

VF = Total volume of filtrate

va = Volume of filtrate analyzed

D = Dilution factor

2.7. Determination of Riboflavin (Vitamin B_2)

The spectrophotometeric method described by Okwu (2004) was used [27]. 5 ml of each of 24 hours inoculums broth was dispensed in 100 ml 5% ethanol solution in distilled water. The mixture was shaken for an hour mechanically and filtered. An aliquot (10 ml) of the filtrate was mixed with an equal volume (10 ml) of 5% potassium permanganate ($KMnO_4$) solution and 10 ml of 30% hydrogen peroxide solution (H_2O) was added to it. The above treatment was also given to a 10 ml portion of standard riboflavin solution as well as a reagent blank. All the flasks (standard, blank and sample) were allowed to stand over a water bath for half an hour and 2 ml of 40% Na_2SO_4 solution was added to each of them. This was made up to 50 ml in a volumetric flask. Their respective absorbance (sample and standard) were measured in a spectrophotometer at 520 nm wavelength. Readings were taken with the regent blank at zero, thus riboflavin content was calculated as:

$$\text{Riboflavin } \mu g/100ml = \frac{100}{V} \times C \times \frac{VF}{va} \times D$$

2.8. Determination of Niacin (Vitamin B_3)

The spectrophotometric method described by [28] was used. A measured volume (5 ml) of each of the 24 hours inoculums broth was treated with 50 ml of 1 N sulphuric acid (H_2SO_4) solution and shaken for 30 minutes. The mixture was further treated with 3 drops of aqueous ammonia mixed well and then filtered. The filtrate was used for the analysis. Standard niacin (nicotinic acid) solution was prepared and diluted as desired. A 10 ml preparation of the standard solution as well as the filtrate and 10 ml of the acid solution (treated with a drop of ammonia) were dispensed into separate flask to serve as standard sample and reagent blank respectively. Each of them was treated with 5 ml of normal potassium cyanide solution and acidified with 5 ml of 0.02 N H_2SO_4 solution, after which its absorbance was read in a spectrophotometer at a wavelength of 470 nm. The regent blank was used to calibrate the instrument at zero. Niacin content was calculated using the formula below:

$$\text{Niacin mg}/100ml = \frac{100}{V} \times \frac{au}{as} \times \frac{C}{1} \times \frac{VF}{va} \times D$$

3. Statistical Analysis

Each experiment was carried out using three replicates and the test results were expressed as means. The statistical analysis was carried out using SPSS software version 21. The significance was determined using ANOVA at a P-value less than 0.05 ($P < 0.05$).

4. Results

4.1. Physiological and Biochemical Characterization of Lactic Acid Bacteria Isolates

The isolates were differentiated on the basis of their morphological, microscopic and biochemical characteristics. Table 1 shows that the isolates were gram positive, catalase negative, oxidase, methyl red, voges proskauer, indole, motility, citrate utilization negative. They grew in 4.5% NaCl; some grew in 6.5% NaCl, at 15˚C and at 45˚C. Carbohydrate fermentation test revealed that all the isolates possessed the ability to ferment glucose, lactose and fructose which fit the classification of lactic acid bacteria as gram positive, catalase negative, oxidase negative.

4.2. Isolation of Lactic Acid Bacteria for Vitamin Production

Table 2 shows that *Lactobacillus plantarum* yielded the highest percentage of occurrence (100%) followed by *Lactobacillus casei* which yielded (75%) occurrence. *Lactobacillus brevis*, *Lactobacillus fermentum* and *Lactococcus lactis* yielded (50%) occurrence each.

Table 1. Morphological and biochemical characteristics of bacterial isolates from selected food sample.

Isolates	1	2	3	4	5
Colony morphology	Moderate growth, circular, raised, milky white colonies, transluscent, moist surface	Moderate growth, milky white, moist surface, raised, smooth, transluscent	Cream white, circular, slightly raised transluscent, smooth colonies, moist surface	Moderate growth, circular, raised, milky white colonies transluscent	Moderate growth, circular moist surface, raised transluscent, milky white coloration.
Gram stain	+	+	+	+	+
Cell arrangement	Oval cells in short chains	Short rods	Short rods	Short rods	Short rods
Catalase Oxidase	−	−	−	−	−
Methyl red	−	−	−	−	−
voges proskauer Indole	−	−	−	−	−
Motility	−	−	−	−	−
Citrate	−	−	−	−	−
4.5% NaCl	+	+	+	+	+
6.5% NaCl	−	+	+	−	−
15˚C	+	+	−	+	+
45˚C	−	−	+	−	−
Glucose	+	+	+	+	+
Fructose	+	+	+	+	+
Lactose	+	+	+	+	+
Mannitol	+	−	−	−	+
Sucrose	+	+	+	+	+
Maltose	−	+	+	+	+
Galactose	+	+	+	+	+
Organism	*Lactococcus lactis*	*Lactobacillus brevis*	*Lactobacillus fermentum*	*Lactobacillus casei*	*Lactobacillus palntarum*

Key: +: positive; −: negative.

Table 2. Percentage occurrence of lactic acid bacteria isolated from different food samples.

Food sample	*Lactococcus lactis*	*Lactobacillus brevis*	*Lactobacillus fermentum*	*Lactobacillus casei*	*Lactobacillus plantarum*
Yoghurt	−	+	+	+	+
Ogi	−	+	−	+	+
Ogiri	+	−	+	+	+
Ugba	+	−	−	−	+
Total	4	4	4	4	4
Number of positive	2	2	2	3	2
% Occurrence	50%	50%	50%	75%	100%

Key: +: positive; −: negative. $\%\ \text{occurrence} = \frac{\text{number of positive}}{\text{Total number}} \times \frac{100}{1}$.

4.3. Determination of the Vitamin Produced

Table 3 shows that *Lactobacillus plantarum* produced highest amount of thiamine (5.8833 µg/100ml), followed by *Lactobacillus fermentum* (5.8167 µg/100ml).

Lactobacillus casei produced (5.6833 µg/100ml) thiamine and *Lactobacillus brevis* produced (5.4967 µg/100ml) while Lactococcus lactis produced (5.4967 µg/100ml) thiamine. *Lactobacillus brevis* produced the least amount of thiamine (5.2267 µg/100ml). Riboflavin is produced highest by *Lactobacillus plantarum* (5.0400 µg/100ml), followed by *Lactococcus lactis* that produced (4.9933 µg/100ml) riboflavin. *Lactobacillus fermentum* produced (4.9533 µg/100ml) riboflavin while *Lactobacillus brevis* produced (4.8833 µg/100ml) riboflavin. *Lactobacillus casei* produced the least amount of riboflavin. Niacin is produced highest by *Lactobacillus casei* (1.6433 µg/100ml) while *Lactobacillus fermentum* produced the least amount (1.2900 µg/100ml). *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus palntarum* produced (1.4533 µg/100ml), (1.3900 µg/100ml) and (1.3833 µg/100ml) niacin respectively.

5. Discussion

The five isolated *Lactobacillus* species showed significant variations in their occurrence pattern in the different fermented food sources (Figure 2 & Figure 3). *Lactobacillus plantarum* had the highest occurrence as it was present in all the

Table 3. Determination of the vitamin produced.

Sample isolate	Thiamine µg/100ml	Riboflavin µg/100ml	Niacin µg/100ml
Lactococcus lactis	$5.2267^{a} \pm 0.30$	$4.9933^{bc} \pm 0.01$	$1.4533^{b} \pm 0.08$
Lactobacillus brevis	$5.4967^{b} \pm 0.01$	$4.9533^{b} \pm 0.01$	$1.3900^{ab} \pm 0.06$
Lactobacillus fermentum	$5.8167^{c} \pm 0.05$	$4.8833^{a} \pm 0.01$	$1.2900^{a} \pm 0.03$
Lactobacillus casei	$5.6833^{bc} \pm 0.04$	$4.8300^{a} \pm 0.04$	$1.6433^{c} \pm 0.06$
Lactobacillus plantarum	$5.8833^{c} \pm 0.01$	$5.0400^{c} \pm 0.06$	$1.3833^{ab} \pm 0.05$

Values show mean of triplicate analysis ± standard deviation (SD). Figures with the same superscript down the column show no significant difference ($P < 0.05$).

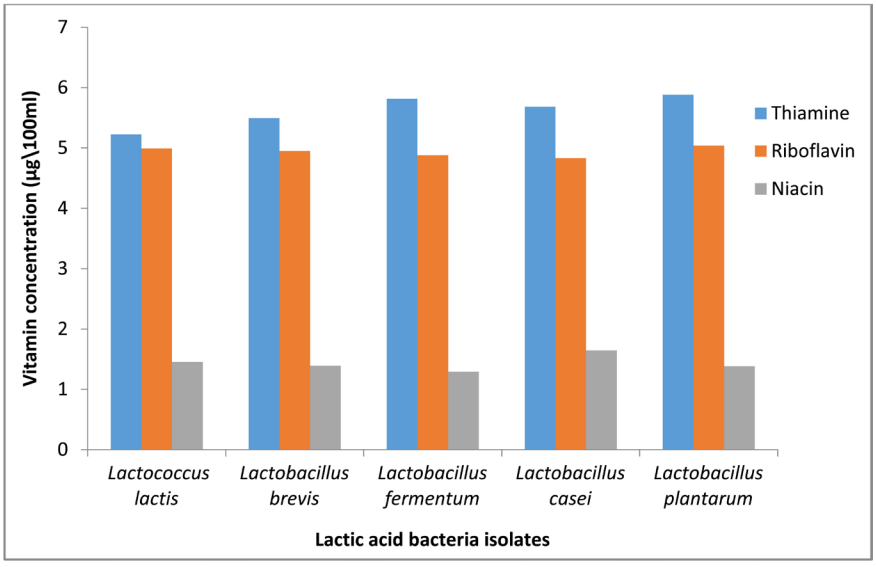

Figure 2. Concentration of the various vitamin produced by LAB isolates.

Figure 3. Pure culture of the LAB isolates stored in MRS agar slant.

test food sources. This is in conformity with the work of [28] where *Lactobacillus plantarum* was identified as the most frequently isolated species in fermented dairy products. *Lactobacillus brevis*, *Lactobacillus fermentum* and *Lactobacillus casei* were not found in Ugba. However, with the exception of *Lactobacillus lactis* all the isolates where present in yoghurt and that made yoghurt the most viable source for isolation of the *Lactobacillus* species as reported by [29] where none of the isolated strains of *Lactobacillus lactis* was found in yoghurt. Lactic acid bacteria were present in fermenting food because of their ability to produce high level of lactic acid as well as being able to survive under high acidic conditions [30]. The identification of different types of lactic acid bacterial species in this study could be due to the fact that majority of the substrate used in the preparation of the fermented food are plant and animal origin and each particular plant species provides a unique environment in terms of competing microorganisms, natural plants antagonist, type, availability and concentration of substances and various physical factors. This condition necessitates for the development of epiphytic flora, from which arises a sequence and population of fermentation microorganisms when the plant materials are harvested and prepared for fermentation [31]. The result of this research work show that all the LAB isolates where able to produce thiamine, riboflavin and niacin, but not in substantial amount which agrees to the work reported by [32] when grown on Vitamin free medium. This finding is an indicative that all the five *Lactobacillus* isolates were able to produce the three water soluble vitamins (thiamin, riboflavin and niacin). But there were variations in the levels of different vitamins produce by different organisms. This finding on the ability of *Lactobacillus* isolates to produce these vitamins was found to agree with report of previous research works [33] [34]. The results also agree with the studies reported of [32] in which all the lactic acid bacteria isolated produced thiamine except *Leuconostoc mesenteriodes* but disagrees with the research work carried out by [35] where

Lactobacillus plantarum did not produce any vitamin in MRS broth and nutrient agar except riboflavin in fish medium which was not in a substantial amount. Thiamine was produced at high concentration than riboflavin and niacin while niacin had the lowest concentration range. Although the variations in the levels of the produced vitamins where significantly different ($P < 0.05$), the result show that *Lactobacillus* isolates possess the potentials for use in biotechnological application as probiotics and as well as industrial application for food enrichment such as in the production of sour dough bread and other products [36]. It was observed that the fermented local foods; yoghurt, Ogi, Ogiri and Ugba are good sources of useful *Lactobacillus* (and possibly) other lactic acid bacteria.

6. Conclusion

The result of this work demonstrates the diversity of lactic acid bacteria in dairy and nondairy fermented foods in Nigeria. The selected fermented foods contain five (5) lactic acid bacteria isolates which were provisionally identified by physiological and biochemical characteristics. All the isolates had potential vitamin (thiamine, riboflavin and niacin) production. *Lactobacillus plantarum* was the best thiamine (5.8833 mg/100ml) and riboflavin (5.0400 µg/100ml) producer while *Lactobacillus casei* had the highest potential for niacin production. The lactic acid bacteria isolated can be used as starter culture with predictable characteristics and contribution to the development of small scale and commercial production of fermented food with vitamins. However, more research work is needed on the optimization of vitamin production from these isolates.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Stamer, J.R. (1979) The Lactic Acid Bacteria: Microbes of Diversity. *Journal Food Technology*, **33**, 60-65.

[2] Wood, B.J.B. and Holzapfel, W.H. (1995) The Genera of LAB, Volume 2. Blackie Academic and Professional, Glasgow.

[3] Hugenholtz, J. (2008) The Lactic Acid Bacterium as a Cell Factory for Food Ingredient Production. *International Dairy Journal*, **18**, 466-475. https://doi.org/10.1016/j.idairyj.2007.11.015

[4] Leroy, F. and Vuyst, L.D. (2004) LAB as Functional Starter Cultures for the Food Industry. *Trend in Food Science and Technology*, **15**, 67-78. https://doi.org/10.1016/j.tifs.2003.09.004

[5] van Hylckama Vlieg, J.E.T. and Hugenholtz, J. (2007) Mining Natural Diversity of LAB for Flavour and Health Benefits. *International Dairy Journal*, **17**, 1290-1297. https://doi.org/10.1016/j.idairyj.2007.02.010

[6] Michaelidoua, A. and Steijnsb, J. (2006) Review: Nutritional and Technological Aspects of Minor Bioactive Components in Milk and Whey: Growth Factors, Vitamins, and Nucleotides. *International Dairy Journal*, **16**, 1421-1426.

https://doi.org/10.1016/j.idairyj.2006.06.018

[7] LeBlanc, J.G., Laiño, J., Juarez del Valle, M., Vannini, V., Van Sinderen, D., Taranto, M., Valdez, G., Savoy, G. and Sesma, F. (2011) B-Group Vitamin Production by Lactic Acid Bacteria-Current Knowledge and Potential Applications. *Journal of Applied Microbiology*, **111**, 1297-1309. https://doi.org/10.1111/j.1365-2672.2011.05157.x

[8] FAO/WHO (2001) Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria.

[9] Ouwehand, A.C., Salminen, S. and Isolauri, E. (2002) Probiotics: An Overview of Beneficial Effects. *Antonie Van Leeuwenhoek*, **82**, 279-289. https://doi.org/10.1023/A:1020620607611

[10] Deshpande, G., Rao, S. and Patole, S. (2011) Progress in the Field of Probiotics: Year 2011. *Current Opinion Gastroenterology*, **27**, 13-18. https://doi.org/10.1097/MOG.0b013e328341373e

[11] Soccol, C.R., de Souza Vandenberghe, L.P., Spier, M.R., Medeiros, A.B.P., Yamaguishi, C.T., Lindner, J.D.D., Pandey, A. and Thomaz-Soccol, V. (2011) The Potential of Probiotics: A Review. *Food Technology and Biotechnology*, **48**, 413-434.

[12] Hugenholtz, J. and Smid, E.J. (2002) Nutraceutical Production with Food-Grade Microorganisms. *Current Opinion in Biotechnology*, **13**, 497-507. https://doi.org/10.1016/S0958-1669(02)00367-1

[13] Pramono, Y.B., Rahayu, E.S., Suparmo and Utami, T. (2008) Isolation and Identification of LAB from Petis a Traditional Fermented Fish. *Jurnal Pengembangan Peternakan Tropis*, **33**, 319-323.

[14] Antara, N.S., Dibia, I.N. and Aryanta, W.R. (2009) Characteristics of LAB Isolated from Bima Mare Milk. *Agritech*, **29**, 1-9.

[15] Lawalata, H.J., Sembiring, L. and Rahayu, E.S. (2011) Molecular Identification of LAB Producing Antimicrobial Agents from Bakasang, an Indonesian Traditional Fermented Fish Products. *Indonesian Journal of Biotechnology*, **16**, 93-99.

[16] Suhartatik, N., Cahyanto, M.N. and Rahayu, E.S. (2014) Isolation and Identification of LAB Producing β-Glukosidase from Indonesian Fermented Food. *International Food Research Journal*, **21**, 973-978.

[17] Purwandhani, S.N. and Rahayu, E.S. (2003) Isolation and Screening of Lactobacillus as Probiotic Agents. *Agritech*, **23**, 67-74. (In Indonesian)

[18] Rahayu, E.S., Yogeswara, A., Mariyatun, Windiarti, L., Utami, T. and Watanabe, K. (2015) Molecular Characteristics of Indigenous Probiotic Strains from Indonesia. *International Journal of Probiotic and Prebiotic*, **10**, 109-116.

[19] Djafaar, T. F., Cahyanto, M.N., Santoso, U. and Rahayu, E.S. (2013) Growth of Indigenous LAB Lactobacillus Platarum-Pentosus T4 and L. Plantarum-Pentosus T35 in Kerandang (*Canavalia virosa*) Milk and Changes of Raffinose. *Malaysian Journal of Microbiology*, **9**, 213-218. https://doi.org/10.21161/mjm.47912

[20] Wikandari, P.R., Suparmo, Marsono, Y. and Rahayu, E.S. (2012) Potency of LAB Isolated from Bekasam as Angiotensin Converting Enzyme Inhibitor Producer on Bekasam Like Product Fermentation. *Agritech*, **32**, 258-264. (In Indonesian)

[21] Djaafar, T.F., Santosa, U., Cahyanto, M.N., Takuya, S., Rahayu, E.S. and Kosuke, N. (2013) Effect of Indigenous LAB Fermentation on Enrichment of Isoflavone and Antioxidant Properties of Kerandang (*Canavalia virosa*) Extract. *International Food Research Journal*, **20**, 2945-2950.

[22] Panagiota, F., Efterpi, C. and Eleftherias, B. (2013) Lactic Acid Bacteria as Source of Functional Ingredients. *Open Science*, **10**, 5772-47766.

[23] De Man, J.C., Rogosa, M. and Sharpe, M.E. (1960) A Medium for the Cultivation of *Lactobacilli*. *Journal of Applied Bacteriology*, **23**, 130-135. https://doi.org/10.1111/j.1365-2672.1960.tb00188.x

[24] Abd, E.L., Gawad, I.A., Abd, E.L., Fatah, A.M. and Al Rubyayyi, K.A. (2010) Identification and Characterization of Dominant Lactic Acid Bacteria Isolated from Traditional Rayeb Milk in Egypt. *American Journal of Science*, **6**, 728-735.

[25] Roissart, H. and Luguet, F.M. (1994) Bacteries lactiques. Aspects fondamentaux et technologiques. Uriage, Lorica, p. 605, by *Lactococcus lactis subsp cremoris* B40. *Carbohydrate Polymer*, **37**, 123-130.

[26] Salminen, S and von Wright, A. (1993) Lactic Acid Bacteria. Marcell Dekker Inc., New York.

[27] Okwu, D.E. (2004) Phytochemical and Vitamin Content of Indigenous Spices of South Eastern Nigeria. *Journal of Sustaining Agriculture Environment*, **46**, 3-10.

[28] Foteini, G.P., Anthoula, A.A., Olga, S.P., George-John, E.N., Nikos, G.C. and Chrysoula, C.T. (2016) Probiotic Potential of Lactic Acid Bacteria from Traditional Fermented Dairy and Meat Products: Assessment by *in Vitro* Tests and Molecular Characterization. *Journal of Probiotics and Health*, **4**, 3.

[29] Sani, S.D and Udeme, J.J.I. (2013) Isolation and Screening of Lactic Acid Bacteria from Fermented Milk Products for Bacteriocin Production. *Annals*. *Food Science and Technology*, **14**, 1.

[30] Cerning, J. (1995) Production of Exopolysaccharides by Lactic Acid Bacteria and Dairy Propioni Bacteria. *Le Lait*, **75**, 463-472. https://doi.org/10.1051/lait:19954-536

[31] Shah, N.P. (2007) Functional Cultures and Health Benefits. *International Diary Journal*, **17**, 1262-1277. https://doi.org/10.1016/j.idairyj.2007.01.014

[32] Misaco, M., Marico, I., Haruka, U., Tae, N., Yuko, S. and Masatsune, M. (2012) Production Potency of Folate, Vitamin B12 and Thiamine by Lactic Acid Bacteria Isolated from Japanese Pickles. *Biological Science*, *Biotechnology and Biochemistry*, **76**, 2061-2067. https://doi.org/10.1271/bbb.120414

[33] Sawase, S.S., Adbul, G.G. and Jadhar, D.S. (2011) Anti-Microbial Activity Associated with Bacteriocin from Lactobacillus Acidophilus. *Journal Resources Antimicrobial*, **1**, 5-8.

[34] Critas, L.M., Casaus, M.P., Huranz, C., Nes, F. and Hernandez, P.E. (2001) Review: Bacteriocins of Lactic Acid Bacteria. *Food Science Technology International*, **7**, 281-305. https://doi.org/10.1106/R8DE-P6HU-CLXP-5RYT

[35] Nguyen, H.K. and Tran, Q.M. (2014) Investigation of Vitamins Production in Lactic Acid Bacteria. A Thesis Submitted to the School of Biotechnology, Vietnam National University-Hochiminh City International University.

[36] Capozzi, V., Menga, V., Digesu, A.M., De vita, P., van suideren, D., Cattirech, L., Fares, C. and Sparo, G. (2011) Biotechnological Production of Vitamin B. Enriched Bread and Pasta. *Journal of Agriculture and Food Chemistry*, **59**, 8013-8020. https://doi.org/10.1021/jf201519h